abcam

ab252930

Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR22421-101
Purification technique	Affinity purification Protein A
Specificity	The Mouse recommendation is based on IP and ChIP results. We do not guarantee WB and IHC for Mouse.
Concentration	0.6 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30

Notes	-

Species	Mouse
Dilution info	1/30
Notes	-

ChIP

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-Fr

Not recommended

Species	Mouse, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/600
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human

Dilution info	1/5000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	-

Target data

See full target information HMGA1

Function	HMG-I/Y bind preferentially to the minor groove of A+T rich regions in double-stranded DNA. It is suggested that these proteins could function in nucleosome phasing and in the 3'-end processing of mRNA transcripts. They are also involved in the transcription regulation of genes containing, or in close proximity to A+T-rich regions.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

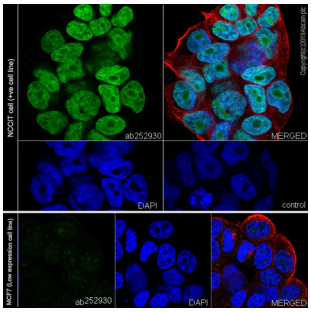
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

11 product images

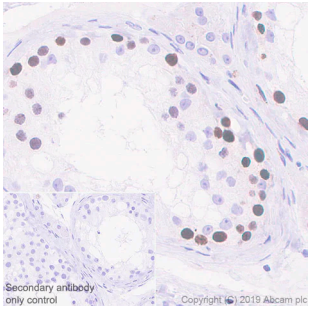


Immunocytochemistry/ Immunofluorescence - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HMGA1 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NCCIT (human pluripotent embryonic carcinoma epithelial cell) cells labeling HMGA1 with ab252930 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in NCCIT cell line. **Low expression cell line**: MCF7 (PMID: 12640109). The nuclear counter stain is DAPI

(blue). Counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).
The negative control is the secondary antibody only.

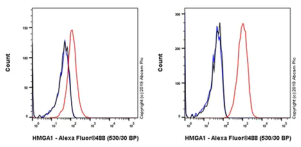


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HMGA1 antibody

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling HMGA1 with ab252930 at 1/5000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in germ cells of human testis (PMID:17935122) is observed. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

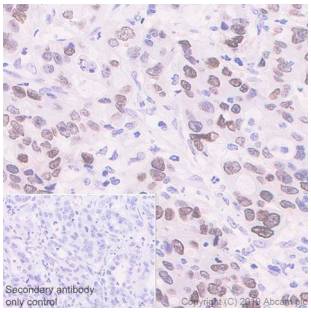
Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



Flow Cytometry (Intracellular) - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Flow Cytometry (Intracellular) staining using rabbit Anti-HMGA1 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized MCF7 (Human breast adenocarcinoma epithelial cell, Left) / NCCIT (Human pluripotent embryonic carcinoma epithelial cell, Right) cell lines labeling HMGA1 with ab252930 at 1/600 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody. **Weak expression control:** MCF7 (PMID:12640109).

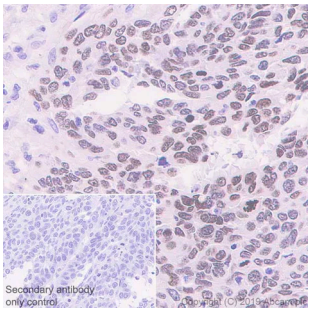


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HMGA1 antibody

Immunohistochemical analysis of paraffin-embedded human pancreas cancer tissue labeling HMGA1 with ab252930 at 1/5000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in human pancreas cancer (PMID: 18473350) is observed. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

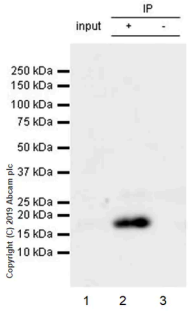


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HMGA1 antibody

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling HMGA1 with ab252930 at 1/5000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in human lung cancer (PMID:25344216) is observed. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunoprecipitation - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

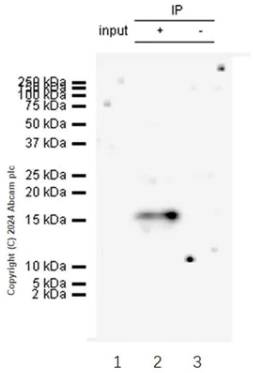
HMGA1 was immunoprecipitated from 0.35 mg HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate with ab252930 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab252930 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used at 1/5000 dilution.
Lane 1: HCT 116 whole cell lysate 10 µg (Input).
Lane 2: ab252930 IP in HCT 116 whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab252930 in HCT 116 whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: 40 seconds.

All lanes:
Immunoprecipitation - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

Predicted band size: 12 kDa

Observed band size: 17 kDa



Immunoprecipitation - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 was immunoprecipitated from 0.35mg of EL4 (mouse lymphoma T lymphocyte) whole cell lysate with ab252930 at 1/30 dilution.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930) at 1/1000 dilution

Lane 1:
EL4 (mouse lymphoma T lymphocyte) whole cell lysate at 10 µg

Lane 2:
ab252930 IP in EL4 (mouse lymphoma T lymphocyte) whole cell lysate at 10 µg

Lane 3:

Rabbit monoclonal IgG (ab172730) instead of ab252930 in EL4 (human epidermoid carcinoma) whole cell lysate at 10 µg

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Predicted band size: 17 kDa

Observed band size: 12 kDa

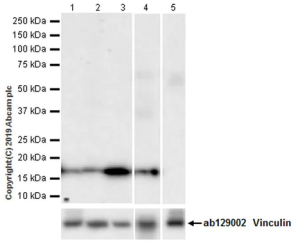
Exposure time: 180s



ChIP - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

Chromatin was prepared from EL4 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab252930 (red) or 5 µg of rabbit normal IgG (gray) was added to 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (sybr green approach).

Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

Blocking buffer and concentration 5% NFDM/TBST
Diluting buffer and concentration 5% NFDM/TBST
The 17-kDa band observed is consistent with what has been described in the literature (PMID:27045138).

Low expression of HMGA1 is expected in the testis (lane 5).

All lanes:
Western blot - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930) at 1/1000 dilution

Lane 1:
HT-1080 (human fibrosarcoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
NCCIT (human pluripotent embryonic carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 3:
HCT116 (human colorectal carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 4:
Human colon cancer tissue lysate at 20 µg

Lane 5:
Human testis tissue lysate at 20 µg

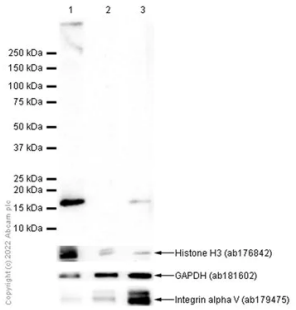
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 12 kDa

Observed band size: 17 kDa

Exposure time: 136s



Western blot - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Western blot staining using rabbit Anti-HMGA1 antibody

Blocking buffer and concentration: 5% NFDM/TBST Diluting buffer and concentration: 5% NFDM/TBST
Lanes 1 - 3:
Western blot - Anti-HMGA1 antibody [EPR22421-101] - BSA and Azide free (ab255277) at 1/1000 dilution

Lanes 1 - 3:
Western blot - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930) at 1/1000 dilution

Lane 1:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) nuclear fraction lysate at 15 µg

Lane 2:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) cytoplasm fraction lysate at 15 µg

Lane 3:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) membrane fraction lysate at 15 µg

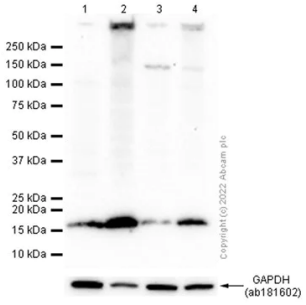
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 12 kDa

Observed band size: 17 kDa

Exposure time: 10s



Western blot - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930)

HMGA1 Western blot staining using rabbit Anti-HMGA1 antibody

Blocking buffer and concentration: 5% NFDM/TBST Diluting buffer and concentration: 5% NFDM/TBST We recommend using 1% SDS Hot lysis prepare method to get desired Western Blot results.
Lanes 1 - 4:
Western blot - Anti-HMGA1 antibody [EPR22421-101] - BSA and Azide free (ab255277) at 1/1000 dilution

Lanes 1 - 4:
Western blot - Anti-HMGA1 antibody [EPR22421-101] - ChIP Grade (ab252930) at 1/1000 dilution

Lane 1:
HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate prepared in RIPA lysis method at 20 µg

Lane 2:
HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate prepared in 1% SDS Hot lysis method at 20 µg

Lane 3:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate prepared in RIPA lysis method at 20 µg

Lane 4:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate prepared in 1% SDS Hot lysis method at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 12 kDa

Observed band size: 17 kDa

Exposure time: 40s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.